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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/00 — A2

(11) International Publication Number: **WO 99/63979**

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/US99/12304

(22) International Filing Date: 3 June 1999 (03.06.99)

(30) Priority Data:
09/093,113 8 June 1998 (08.06.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: INHIBITION OF Δ-9-DESATURASE ACTIVITY BY SAPONINS

(57) Abstract

Compositions comprising a saponin or a saponin metabolite, such as dietary supplements and nutritional solutions, are described for use as inhibitors of Δ-9-desaturase enzyme. Methods for inhibiting the activity of Δ-9-desaturase enzyme and methods for treating obesity, diabetes and atherosclerosis are also described.

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INHIBITION OF Δ-9-DESATURASE ACTIVITY BY SAPONINS

BACKGROUND OF THE INVENTION

Consumption of diets supplemented with alternate precursor fatty acids or substrates which influence arachidonic acid metabolism (Carrick *et al., Shock 2*:421-426 (1994); Yacoob and Calder, *Cell Immunol. 163*:120-128 (1995)) can decrease the production of proinflammatory mediators (such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, prostaglandin (PG) E_2, and thromboxane (Tx)A_2) during infection or inflammation, including some disease conditions (Lowry, S., *Arch Surg* 128:1223-41 (1993); Klosterhalfen, B., *et al., Circul Shock* 35:237-244 (1991)). Polyunsaturated fatty acids (PUFA) such as α-linolenic acid (LA: 18:3 ω3), eicosapentaenoic acid (EPA: 20:5ω3) dihomo-γ-linolenic acid (20:3ω6) or docosahexaenoic acid (DHA:22:6ω3) have been employed as preventive and therapeutic modalities in controlling inflammatory responses in experimental animals models (Carrick *et al., Shock 2*:421-426 (1994); Yacoob and Calder, *Cell Immunol. 163*:120-128 (1995)) and in clinical trials (Espersen *et al., Clin. Rheumatol. 11*:393-395 (1992); Engler *et al., J. Hyperten 10*:1197-1204 (1992); Harrobin, *Rev. Contem. Pharmacolther 1*:1-45 (1990)).

Saponins, a heterogeneous mixture of chemically distinguishable triterpenoid or steroidal glycosides, are present in many plants and vegetables, and are generally well tolerated when consumed as foods (Marston *et al., J. Ethnopharmacol 38*:215-223 (1993); Okenful, *Food Chem. 6*:19-40 (1981)). Moreover, orally administered saponins increase absorption of other nutrients and experimental oral vaccines into the circulation (Campbell and Peerbaye, *Res. Immunol 143*:526-530 (1992). When consumed as supplements along with dietary fats, saponins possessing antioxidant properties can prevent fatty acids from being oxidized (Tsujino *et al., Biosc. Biotchnol. Biochem. 58*:1731-1732 (1994)). Further, the ability of saponins to form stable emulsions (Okenful, *Food Chem. 6*:19-40 (1981)) may allow their absorption over a longer period. Thus, saponins can increase uptake and incorporation of precursor fatty acids into the membrane phospholipids and may, consequently, affect

arachidonic acid metabolism. Crude saponins, along with the semi-purified fractions (*Quil A* and Quillayanin), from the bark of the South American plant *Quillaja Saponaria*, and the ginsenosides from ginseng root, consist of triterpenoid saponins which have been shown to possess antioxidant, emulsifying, and immunopotentiating properties (Campbell and Peerbaye, *Res. Immunol. 143*:526-530 (1992); Tsujino *et al., Biosc. Biotechnol. Biochem. 58*:1731-1732 (1994); Dalsgaard, *Arch fur ges. Virusfor. 44*:243-254 (1974)).

SUMMARY OF THE INVENTION

As described herein, the effects of feeding diets enriched with 15% safflower oil (SO), in the presence or absence of one or more saponins, on the membrane fatty acid composition of various tissues, and also on the production of dienoic eicosanoids and cytokines in response to an intraperitoneal injection of a lethal dose of lipopolysaccharide (LPS), were investigated. In mice fed Quil A-supplemented diets, the tissue levels of oleic acid were significantly lower than in controls, which indicates that these saponins or their metabolites inhibit the activity of Δ-9 desaturase enzyme. Further, the arachidonic acid levels were markedly lower in animals fed saponin-supplemented SO diets compared to those fed SO alone. Consistent with these observations, the circulating levels of PGE_2 and TxB_2 produced in response to an intraperitoneal injection of LPS were markedly lower in mice maintained on Quil A-supplemented diets.

Additionally, in mice fed Quil A, the plasma levels of the anti-inflammatory interleukin (IL)-10 were significantly elevated, and those of the proinflammatory IL-12 were markedly reduced. In mice fed SO diets supplemented with 1% ginseng saponins, stearic acid levels were markedly higher with a concomitant decrease in the levels of oleic acid, and circulating levels of TNF-α in response to LPS were significantly reduced. These data indicate that saponins having the ability to decrease the activity of Δ-9 desaturase enzyme, and to lower the production of proinflammatory mediators, can be used as dietary supplements for enteral and parenteral nutrition.

The invention pertains to compositions comprising one or more saponins in an amount effective to inhibit Δ-9 desaturase enzyme. In particular embodiments, the composition is a dietary supplement or nutritional solution, such as a dietary supplement or nutritional solution suitable for enteral or parenteral administration. In one embodiment of the invention, the saponin of the composition is essentially

purified. In a particular embodiment, the saponin is selected from the group consisting of crude saponin extracts, semi-purified saponin fractions (such as Quil A and Quillayanin), purified saponin extracts, and ginseng saponins. In particular embodiments, the saponin is derived from *Quillaja saponaria*, *Panax trifolium*, *Panax quinquefolium* and *Glycyrrhiza glabra*. In other embodiments, the composition further comprises essential fatty acids and/or essential vitamins and minerals.

The invention further relates to a dietary supplement or medical food comprising an effective amount of a saponin. For example, the dietary supplement or medical food can be selected from the group consisting of nutritional beverage, baked good (cookie, brownie, fudge, cake, bread, biscuit and cracker), pudding, confection, snack food, ice cream, frozen confection, and non-baked, extruded food product such as a bar.

The invention also pertains to a method of inhibiting Δ-9 desaturase enzyme activity in a mammal comprising administering a composition comprising an effective amount of one or more saponins to a mammal in need thereof. In one embodiment, the composition to be administered is a dietary supplement or nutritional solution, such as one which is suitable for enteral or parenteral administration. In another embodiment, the composition further comprises essential fatty acids and/or essential vitamins and minerals. The composition can be administered enterally or parenterally.

The invention also pertains to a method of inhibiting Δ-9 desaturase enzyme activity in a mammal comprising administering a composition comprising an effective amount of a saponin metabolite to a mammal in need thereof, as well as to compositions comprising a saponin metabolite in an amount effective to treat inflammation.

The invention further relates to a method of inhibiting palmitic acid or stearic acid metabolism in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite. Inhibition of stearic acid metabolism results in inhibition of the formation of oleic acid and oleic acid metabolites, such as PGE_2 and thromboxane $(Tx)B_2$. The invention also relates to a method of inhibiting the formation of oleic acid in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite.

The invention further relates to a method of inhibiting the level of PGE_2, PGE_1, PGE_{1+2}, TxB_2 or proinflammatory cytokines such as IL-12 in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite.

The invention also relates to a method of enhancing the level of anti-inflammatory cytokines such as IL-10 in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite.

Saponins have several benefits and advantages for the health of mammals to which it is administered. In general, consumption of saponin-supplemented diets can improve the functions of vital organs such as heart, lungs, liver and kidneys by increasing the absorption of nutrients by the body. The levels of TNF, a proinflammatory mediator, are not elevated in mice fed saponins in contrast to TNF levels with other anti-inflammatory drugs; therefore, use of saponins as anti-inflammatory agents does not induce the undesirable side effects induced by many other anti-inflammatory agents. Proinflammatory mediators such as PGE_2 and IL-6 are also associated with increased mortality of patients with cancer/neoplasia and of those with sepsis and septic shock. The ability of saponins to decrease the levels of one or more of these mediators without affecting the levels of TNF can positively impact therapy regimens.

BRIEF DESCRIPTION OF THE DRAWINGS

The Figure is a schematic diagram depicting the precursors and products of Δ-9-desaturase enzyme.

DETAILED DESCRIPTION OF THE INVENTION

An elevation in the levels of proinflammatory mediators such as PGE_2, TxB_2, and TNF-α, IL-6 and IL-12 is associated with severity of septic shock and plays a major role in the pathogenesis of sepsis. Attempts to ameliorate the production of these mediators have become an important strategy in the management of the critically ill patients with septic shock and other inflammatory diseases. The ability of saponins to increase absorption of precursor fatty acids could affect the tissue levels of arachidonic acid and subsequently alter the production of prostaglandins and thromboxanes which play a major role during infection/inflammation.

As illustrated in the Figure, palmitic acid is converted to palmitoleic acid and stearic acid is converted to oleic acid by the activity of a Δ-9-desaturase enzyme. An increase in the oleic acid/stearic acid ratio is a common finding in several clinical conditions such as obesity, non-insulin dependent diabetes mellitus (Pan *et al., J Nutr 124*:1555-1565 (1994); Wahle *et al., Comp Biochem Physiol 109*:235-244 (1994); Pan *et al., J Clin Invest 96*:2802-2808 (1995)), and atherosclerosis. Work described herein indicates that consumption of diets supplemented with saponins, e.g., *Quil A* or other structurally similar saponins, can decrease the ratio of oleic acid to stearic acid which may result from an inhibition in the activity of Δ-9 desaturase enzyme. Therefore, saponins, through their ability to decrease the activity of Δ-9 desaturase enzyme, can be useful as dietary supplements in enteral and parenteral nutrition to provide a wide array of beneficial effects by ameliorating symptoms associated with inflammatory conditions and diseases such as diabetes, obesity and atherosclerosis. In addition, saponins alone or along with selected dietary fats could be mixed with oral vaccines to form stable emulsions to optimize immune responses for a specific infection in mammals, e.g., humans, while decreasing the formation of proinflammatory mediators. Further, consumption of saponins results in a significant improvement of the vital organ functions, where as administration of nonsteroidal anti-inflammatory drugs result in several serious side effects such as ulceration and liver damage. Moreover, all these beneficial effects are exerted in as short a period as 3-4 days, whereas other agents take as many as 6-7 days to benefit patients even after tube feeding.

The ability of nutrients to modulate the production of cytokines (Carrick *et al., Shock 2*:421-426 (1994); Yacoob and Calder, *Cell Immunol 163*:120-128 (1995)) associated with the severity of sepsis (Lowry, *Arch Surg 128*:1223-41 (1993)) has been explored (Utsunomiya *et al., Biochim Biophys Acta 1214*:333-339 (1994); Clouva-Molyvdas *et al., J Parent En. Nutur 16*:343-347 (1992)), and the results are often contradictory. Endogenous IL-6 plays a crucial role during sepsis (Damas *et al., Ann Surg 215*:356-362 (1991); Starnes, Jr., *et al., J Immunol 145*:4185-4191 (1990)). In the studies described herein, the levels of IL-6 were unaffected in mice fed *Quil A* supplemented diets.

Quillaja saponins, when administered orally (Chavali and Campbell, *Immunobiol 174*:347-359 (1987); Chavali and Campbell, *Int Arch Allergy Immunol 84*:129-134 (1987)) or parenterally (Chavali *et al., Clin Exp Immuol 74*:339-343 (1988)), can augment the production of antibodies to many experimental vaccines

and protect animals against infection (Campbell and Peerbaye, *Res Immunol 143*:526-530 (1992)). Thus, the demonstrated ability of saponins to potentiate immune responses (Campbell and Peerbaye, Res *Immunol 143*:526-530 (1992); Chavali and Campbell, *Immunobiol 174*:347-359 (1987); Chavali and Campbell, *Int Arch Allergy Immunol 84*:129-134 (1987); Chavali *et al., Clin Exp Immuol 74*:339-343 (1988)) is explained if IL-10 production is augmented with a concomitant decrease in IL-12 levels, as demonstrated in mice fed Quil A-supplemented diets (Table 3). Interleukin 10 is an anti-inflammatory cytokine which influences differentiation of both T and B cells (Burdin *et al., J Immunol 154*:2533-2544 (1995); Appelberg *et al., Immunol 82*:361-364 (1994); Ming *et al., Clin Exp Immunol 89*:148-153 (1992)), confers protection against infection and enhances humoral immunity through favoring T helper type 2 cell responses (van der Poll *et al., J Immul 158*:1971-1975 (1997); Huhn *et al., Clin Pharmacol Ther 62*:171-180 (1997)). In sharp contrast, IL-12 is a pro-inflammatory mediator which can suppress humoral immune responses, induce synthesis of TNF-α and IFN-γ and favor T helper type 1 cell responses (Houssiau *et al., Clin Exp Immunol 108*:375-380 (1997); Pearlman *et al., J Immunol 154*:4658-4664 (1995)). Interleukin-10 decreases LPS-induced production of TNF-α from macrophages (Gerard *et al., J Exp Med 177*:547-550 (1993)), from the whole blood (Marchant *et al., Prog Clin Biol Res 388*:417-423 (1994)), and in mice (Standiford *et al., J Immunol 155*:2222-2229 (1995)). Further, endogenous IL-10 is elevated as a protective mechanism in animals injected with LPS (Standiford *et al., J Immunol 155*:2222-2229 (1995)). Moreover, endotoxin induced lethality is increased in IL-10 deficient mice (Berg *et al., J Clin Instest 96*:2339-2347 (1995)). Elsewhere, evidence suggests that consumption of *Quil A* saponins enhances the production of endogenous IL-10 and confers protection during endotoxic shock (Chavali *et al., Int Arch Allergy Immunol 114*:153-160 (1997)). Further, it has been shown that *Quillaja* crude saponins enhance the production of IL-10 in mice (Tadokoro *et al., Immunol 89*:368-374 (1996)).

It has been reported that an increase in Δ-9 desaturase activity is associated with obesity, atherosclerosis and diabetes mellitus. Therefore, based on data shown herein, it is reasonable to suggest that saponins having the ability to decrease the activity of Δ-9 desaturase enzyme, and to lower the production of proinflammatory mediators, can be used as dietary supplements for enteral and parenteral nutrition for patients with obesity, atherosclerosis and diabetes mellitus. Further, consumption of

such dietary supplements may be of immense prophylactic value even for the general public.

Thus, the invention encompasses compositions comprising saponin or a saponin metabolite in an amount effective to inhibit (e.g., reduce or abolish) Δ-9 desaturase enzyme activity. Such compositions can be used in the treatment or inhibition of obesity, atherosclerosis and diabetes mellitus and related conditions. As used herein, treatment or inhibition encompasses reduction in symptomology associated with a particular disorder, including complete resolution of the condition. Treatment and inhibition are also intended to include reduction or minimization of risk of the condition in a mammal at risk for such symptoms or conditions.

Compositions comprising saponins or a saponin metabolite can be in any form suitable for administration to a mammal, including tablet, powder, capsule, liquid, injectable and suppository forms. In preferred embodiments, the composition is a dietary supplement or a nutritional solution. For example, the dietary supplement can contain essential fatty acids and/or essential vitamins and minerals in addition to saponins or saponin metabolites. Saponins can also be used along with other dietary fats such as sesame seed oil, fish oil, or linseed oil. Such mixtures of saponins and dietary fats can be consumed as dietary supplements or as essential ingredients in consumable foods and drinks. The dietary supplement can be provided in a variety of forms, such as nutritional beverages, baked goods (e.g., cookies, brownies, fudge, cake, breads, biscuits, crackers), puddings, confections (i.e., candy), snack foods (e.g., pretzels, chips), ice cream, frozen confections and novelties, or non-baked, extruded foods such as bars.

The dietary supplement can provide optimal nutrition for growth and weight maintenance, and can comprise protein, carbohydrate and fat components, alone or in combination, in addition to an effective amount of one or more saponins or saponin metabolites. For example, the carbohydrate sources can include, but are not limited to, one or more of corn syrup, high fructose corn syrup, corn starch, maltodextrin, fructose, lactose, glucose, sucrose, dextrose and maltose. The protein sources can include, but are not limited to, one or more of whey protein, whey protein concentrate, whey powder, egg protein, soy protein, soy protein isolate and caseinate. The fat sources can include, but are not limited to, one or more of dietary fats, coconut oil, peanut oil, safflower oil, canola oil, corn oil, sesame seed oil, fish oil and vegetable oil, as well as structured triglycerides, long-chain triglycerides and medium-chain triglycerides. The dietary supplement can also comprise adjunct

ingredients such as emulsifiers (*e.g.* saponins), preservatives, artificial sweeteners, thickeners, colorings and flavors which improve the palatability, stability, shelf-life and organoleptic properties of the composition (see, for example, U.S. Patent Nos. 5,674,853 and 5,397,778).

The nutritional solution can be a parenteral nutritional solution, such as a total parenteral nutritional solution which contains all essential nutrients for health. The composition can also comprise additional components as appropriate. For instance, the saponin or saponin metabolite can be formulated with a physiologically acceptable medium to prepare a pharmaceutical composition. The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The saponin or saponin metabolite can also be formulated in a vaccine composition.

As used herein, an effective amount includes an amount sufficient to show statistically significant anti-inflammatory effects. The range of effective amounts will generally be from about 0.1 to about 10 mg/kg body weight of the mammal to be treated. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known in the art, and will depend on the ultimate pharmaceutical formulation desired. Saponins or saponin metabolites can be present in the composition in a purified form or administered in the form of a crude or semi-purified extract. In particular embodiments, the saponin is selected from the group consisting of crude saponin extracts, semi-purified saponin fractions (such as Quil A and Quillayanin), purified saponin extracts, and ginseng saponins. In particular embodiments, the saponin is derived from *Quillaja saponaria*, *Panax trifolium*, *Panax quinquefolium* and *Glycyrrhiza glabra*.

As used herein, saponins can be in either an isolated or synthetic form; that is, saponin can be isolated from a natural plant source or it can be synthesized chemically. Moreover, the term "saponin" is intended to include saponin metabolites as well as a saponin itself, as well as combinations of one or more saponins or saponin metabolites. Saponin metabolites include any secondary metabolite produced by direct or subsequent metabolism of a saponin; that is, saponin metabolites include products produced by direct metabolism of a saponin itself (primary metabolites), as well as secondary products produced by further metabolism of the primary metabolites (secondary metabolites). The determination

of the metabolite or metabolites responsible for the Δ-9 desaturase inhibiting properties of a saponin can be determined by assessing the ability of each saponin metabolite to inhibit Δ-9-desaturase activity by art recognized methods such as those described herein or by methods such as those described by Shimizu *et al.* (*Lipids* 26:512-516 (1991)). Saponin metabolites which are identified as having inhibitory ability *in vitro* can then be studied to assess the *in vivo* anti-inflammatory properties of the metabolite by art recognized methods such as those described herein or those described by Shimizu *et al.* (*Lipids 26*:512-516 (1991)). Compounds which are structurally related to saponins and metabolic products thereof, such as ginsenosides, can also be used in the methods described herein.

The invention also relates to methods of treating obesity, atherosclerosis or diabetes, or inhibiting Δ-9 desaturase activity by administering a composition comprising an effective amount of a saponin to a mammal in need thereof. Suitable mammals include, but are not limited to, primates (e.g., humans), dogs, cats, cows, horses, pigs, goats and rodents (e.g., rats, mice and hamsters). Methods of administering such compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral, suppository and intranasal. Particularly preferred methods of administration are enteral and parenteral administration. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release devices. The compositions of this invention can also be administered as part of a combinatorial therapy with other agents, including anti-inflammatory agents and antibiotics.

The methods of the present invention can also be used to reduce the incidence or symptomology of inflammation associated with infection by various organisms, as well as to reduce the occurrence or severity of inflammation associated with other conditions. For example, the methods of the present invention are useful to treat conditions such as arthritis, lyme disease, aging, breast cancer, head and neck cancer, common colds and flu and sepsis, as well as any other conditions in which a reduction of Δ-9 desaturase activity is desirable.

The invention also encompasses methods of inhibiting Δ-9-desaturase activity in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite. Inhibition of Δ-9-desaturase activity is intended to include an inhibition or reduction in levels or activities of enzymes responsible for the Δ-9-desaturation of stearic acid, such as Δ-9-desaturase enzyme. The inhibition of Δ-9-desaturase activity results in an increase

in the level of stearic acid and palmitic acid, and a decrease in any or all of the compounds for which oleic acid or palmitoleic acid is a precursor. One result of Δ-9-desaturase inhibition is a decrease in proinflammatory mediators such as prostaglandins. Thus, the invention also encompasses a method of inhibiting the level of PGE_2 , PGE_1 or PGE_{1+2} in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite.

Moreover, even in the absence of effects on arachidonic acid levels, a reduction in PGE_2 suggests that saponins have the ability to inhibit or decrease the level or activity of PLA_2, which is responsible for the release of arachidonic acid from membrane phospholipids. Thus, the invention also relates to a method of inhibiting the activity of PLA_2 in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin or a saponin metabolite.

The following Examples are offered for the purpose of illustrating the present invention and are not to be construed to limit the scope of this invention. The teachings of all references cited herein are hereby incorporated herein by reference.

EXAMPLES

EXAMPLE 1

MATERIALS AND METHODS

Animals

Six-8 week old, inbred, female Balb/c mice (Taconic Farms, Germantown, NY) were maintained in an approved animal facility with 12 hour day and 12 hour night cycle. They were allowed free access to drinking water and the experimental diets were fed daily at dusk.

Diets

The AIN-76A fat-free powder along with 0.05%t-butyl hydroxy toluene, an antioxidant, was mixed with 5 wt% (10% Kcal) of safflower oil (Oilseeds International Ltd., Fresno, CA), partitioned into daily rations packaged in separate whirl-pack bags, flushed with N_2, and stored at 4°C. Where mentioned, these diets

were supplemented with 0.25% saponin (*Quil A*) obtained from Superfos Biosector a/s, Denmark.

Fatty Acid Analysis

The liver tissues (100 mg) were homogenized and extracted with chloroform:methanol (2:1 v/v) solvent mixture containing 0.01% *t*-butylated hydroxytoluene as an antioxidant (Folch *et al., J. Biol. Chem. 226*:497-509 (1957)). The chloroform fractions were evaporated to dryness under N_2 and reconstituted in the same solvent. The total phospholipids were separated by thin-layer chromatography on silica gel-H plates (Analtech Inc. Newark, DE), and the fatty acid methyl esters were derived (Metcalfe and Schmitz, *Anal. Chem. 33*:363-364 (1961)) and analyzed on a fused-silica capillary column (100 m, 0.25 mm ID, 0.20 μm thickness; SP™ -2560, Supelco Inc., Bellefonte, PA) using a gas chromatograph (5890 Series II) equipped with a mass selective detector (5971, Hewlett-Packard). The results were expressed as relative percent of identified fatty acids on a molar basis, using heptadecaenoic acid (17:0) as an internal standard.

Endotoxin-induced *in vivo* production of cytokines

Using heparinized syringes, blood samples from mice were collected from the inferior *vena cava* at 90 minutes and 3 hours after an intraperitoneal injection of a lethal dose (LD_{50}/24h=20mg/kg) of lipopolysaccharide (LPS: B55:05, Difco Laboratories, Detroit, MI). This protocol has been approved by the Harvard Medical Area Standing Committee on Animals. Plasma levels of TNF-α, interleukin (IL)-6, IL-10 and IL-12 were determined using enzyme-linked immunosorbent assay kits (Biosource International, Camarillo, CA).

Radioimmunoassays for Prostaglandin (PG)E_2, and Thromboxane (TX) B_2

An aliquot (50 µl) of plasma was diluted in 1 ml PBS, extracted twice with ethyl acetate (2 ml each), and the solvent fractions were pooled and then evaporated to dryness under N_2. The resultant extract was resuspended in PBS containing 0.1% gelatin, and the levels of PGE_2 and TXB_2 were determined in a radioimmunoassay according to the procedures described elsewhere (Granstrom and Kindahl, *Adv Prost Throm Res 5*:119-210 (1978)). The rabbit anti-PGE_2 and anti-TXB_2 antibodies were purchased from Perseptive Diagnostics (Cambridge, MA). According to the

supplier's technical information, the PGE_2 antiserum has a 50% cross-reactivity with PGE_1. Therefore, the actual amount of PGE_2 reported may represent up to a maximum of 50% PGE_1, if present in the samples. No effort was made to correct for cross-reactivity with PGE_1, and the results are referred to as PGE_{1+2}.

Statistical Analysis

The significance in differences in the mean concentrations of cytokines, eicosanoids, and the tissue levels of fatty acids was determined using a student's t-test. A *P* value of less than 0.05 was considered significant.

RESULTS

Alterations in the fatty acid composition (mean ± s.d. molar %) of stearic acid, oleic acid and arachidonic acid (AA) were determined in the membrane phospholipids of livers from mice fed SO diets supplemented with Quil A saponins, and the data are summarized in Tables 1 and 2. The tissue levels of oleic acid were significantly lower (p,0.05) in mice maintained on Quil A supplemented SO diets compared to those fed SO alone (Table 1). Data represent the molar percents of oleic acid (OA), stearic acid (SA) and arachidonic acid (AA) in the liver membrane phospholipids. Similar data were obtained in the animals fed saponin-supplemented diets containing other dietary fats such as sesame seed oil, menhaden fish oil, or linseed oil.

Alterations in the membrane fatty acid composition (Tables 1) could affect AA metabolism (Harrobin, *Rev Contem* Rharmacolther *1*:1-45 (1990); Weaver and Holob, *Prog Food Nutr Sci 12*:111-150 (1988)). Thus, the levels of LPS-induced production of PGE_2 and TXB_2 in mice fed *Quil* A-supplemented SO diets were determined, and the data are summarized in Table 2. Compared with animals fed SO diets alone, in mice fed Quil A-supplemented diets, the LPS-induced production of PGE_2 (364±41 (SO) *vs.* 280±39 (SO+) pg/ml) and TxB_2 (67±15 *(SO) vs.* 49±26 (SO+) pg/ml) were markedly lower (p<0.05).

During infection and inflammation (Lowry, *Arch Surg 128*:1223-41 (1993); Klosterhalfen *et al., Circul Shock 35*:237-244 (1991)), both prostaglandins (Zhong *et al., Immunol 84*:446-452 (1995); Pruimboom *et al., Immunol Lett 41*:255-260 (1994); Hilger *et al., Int Arch Aller Immunol 107*:383-384 (1995)) and fatty acids (Carrick *et al., Shock 2*:421-426 (1994); Yacoob and Calder, *Cell Immunol 163*:120-128 (1995)) affect the production of cytokines. Thus the plasma levels of TNF-α,

IL-6, IL-10, and IL-12 produced in response to an intraperitoneal injection of LPS were determined (Table 3). Consumption of Quil A-supplemented SO diets caused a significant increase ($p<0.05$) in the amounts (pg/ml) of anti-inflammatory IL-10 (414±67) compared to those fed SO alone (231±30) in response to a lethal dose (20 mg/kg) of LPS. In contrast, the levels of pro-inflammatory IL-12 (pg/ml) were markedly lower ($p<0.05$) in the Quil A group (15±1) compared to the SO group (19±1). The concentrations of TNF-α and IL-6 in both the dietary groups did not differ significantly.

Table 1:

Diets	SA	OA	AA
Safflower oil(SO)	25±1	4.5±0.5	26.1±1
SO+Quil A (SO+)	26±1	3.4±0.4*	23.8±1*

Table 2:

Diets	PGE_2	TxB_2
Safflower oil (SO)	364±41	67±15
SO+Quil A (SO+)	280±39*	49±26*

Table 3:

Diets	IL-10	IL-12
Safflower oil (SO)	231±30	10±1
SO+Quil A (SO+)	414±67*	15±1*

*Level of significance $P<0.05$

EXAMPLE 2: DIETARY SUPPLEMENTATION OF GINSENG SAPONINS DECREASE Δ-9 DESATURASE ACTIVITY AND REDUCE THE PRODUCTION OF TUMOR NECROSIS FACTOR-α IN RESPONSE TO ENDOTOXIN

The effects of feeding diets enriched with 5% safflower oil (SO) supplemented with 1% ginseng (*Panax quinquefolium)* saponins on the fatty acid composition of the liver were determined. The stearic acid levels in the livers from mice fed ginseng saponin-supplemented diets were markedly higher ($p<0.01$), with a concomitant decrease in the levels of oleic acid. Consequently, the oleic acid/stearic acid ratio was significantly lower ($p<0.01$) in animals fed ginseng-supplemented diets compared to those fed SO alone, which suggests that consumption of ginseng saponins inhibited the Δ-9 desaturase enzyme activity.

Table 4:

Diet	SA	OA	OA/SA
Safflower oil (SO)	20.3±0.8	7.8±0.4	0.4±0.03
SO + Ginseng Saponins (SO+)	22.1±0.6	6.5±0.6	0.3±0.03

The effects of feeding diets enriched with 5% SO supplemented with 1% ginseng on the production of tumor necrosis factor (TNF)-α in response to an intraperitoneal injection of lipopolysaccharide (LPS:0.5 mg/kg) were determined. The circulating levels of TNF-α in response to LPS were significantly reduced ($P<0.01$) in mice fed a diet supplemented with ginseng saponins compared to those fed a SO diet alone.

Similar results were obtained in animals fed diets supplemented with crude saponin preparations from the plants *Quillaja saponaria* and *Glycyrrhiza glabra* (liquorice).

Table 5:

Diet	Tumor Necrosis Factor(TNF)-α (mean ± SE; pg/ml)
Safflower Oil (SO)	9,448±1,663
SO + Ginseng saponins	2,889±331

EQUIVALENTS

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the claims.

CLAIMS

What is claimed is:

1. A composition comprising a saponin in an amount effective to inhibit Δ-9 desaturase enzyme activity.

2. A composition according to Claim 1 wherein the composition is a dietary supplement or nutritional solution.

3. A composition according to Claim 1 wherein said saponin is essentially purified.

4. A composition according to Claim 1 wherein said saponin is derived from a source selected from the group consisting of *Quillaja saponaria*, *Panax trifolium*, *Panax quinquefolium* and *Glycyrrhiza glabra*.

5. A composition according to Claim 1 further comprising essential fatty acids.

6. A composition according to Claim 1 further comprising essential vitamins and minerals.

7. A method of inhibiting Δ-9 desaturase enzyme activity in a mammal comprising administering a composition comprising an effective amount of a saponin to a mammal in need thereof.

8. A method according to Claim 8 wherein the composition is a dietary supplement or nutritional solution.

9. A method according to Claim 8 wherein said saponin is essentially purified.

10. A method according to Claim 8 wherein said saponin is derived from a source selected from the group consisting of *Quillaja saponaria*, *Panax trifolium*, *Panax quinquefolium* and *Glycyrrhiza glabra*.

11. A method according to Claim 8 wherein said composition further comprises essential fatty acids.

12. A method according to Claim 8 wherein said composition further comprises essential vitamins and minerals.

13. A method of inhibiting Δ-9 desaturase enzyme activity in a mammal comprising administering a composition comprising an effective amount of a saponin metabolite to a mammal in need thereof.

14. A method of inhibiting the formation of oleic acid or palmitoleic acid in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin.

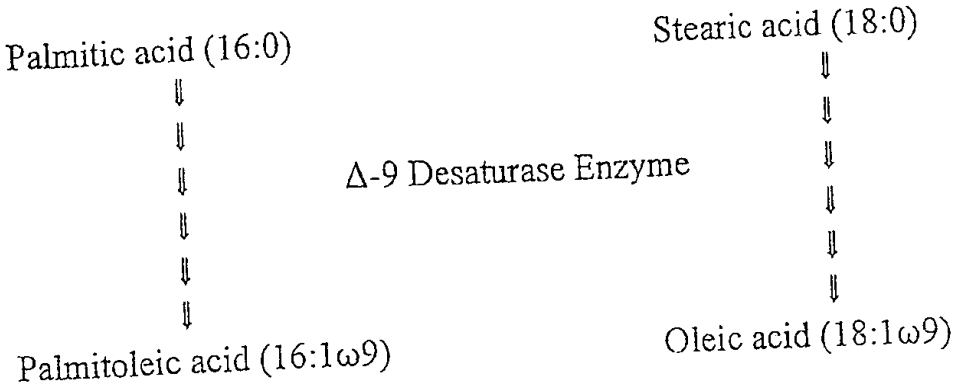
15. A method of inhibiting the formation of arachidonic acid metabolites in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin.

16. A method of inhibiting the level of PGE_1, PGE_2 or $PGE_{1,2}$ in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin.

17. A method of treating obesity in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin, thereby inhibiting delta-9 desaturase enzyme activity.

18. A method of treating diabetes mellitus in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin, thereby inhibiting delta-9 desaturase enzyme activity.

19. A method of treating atherosclerosis in a mammal comprising administering to the mammal a composition comprising an effective amount of a saponin, thereby inhibiting delta-9 desaturase enzyme activity.


Palmitic acid (16:0)
Stearic acid (18:0)
Δ-9 Desaturase Enzyme
Palmitoleic acid (16:1ω9)
Oleic acid (18:1ω9)